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Fungal Viruses

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"There was never any question in our minds but that we were dealing with a virus. I tried before 1950 to interest various virologists in the disease and to the study of it, without avail."

James W. Sinden, 1967

INTRODUCTION

The fungi represent a heterogeneous assemblage of eukaryotic microorganisms. Fungal metabolism is characteristically heterotrophic, and the vast majority of fungi are filamentous, haploid organisms reproducing either sexually or asexually through spores (2-4). Several of the fungi have proven to be excellent experimental systems, and among these are species adapted for formal genetic analysis (38, 71, 75).

Viruses are now recognized as common in fungi and indeed have been reported in species representing each of the major taxonomic classes of the fungi. Clearly, the presence of viruses in fungi adds a new dimension to experimental mycology insofar as these viruses may influence profoundly the metabolism and genetics of the fungal cell.

It is our intention to review here all of the data available on fungal viruses and to give special attention to the biological implications of an association between fungus and phage. It is not, however, our aim to review in detail the transmission of nonfungal viruses by fungal vectors. This subject has been reviewed extensively by others (85, 99).

DISTRIBUTION AND DISCOVERY OF FUNGAL VIRUSES

Viruses have been reported to occur in over 60 species from some 50 genera of fungi (Table 1). Most of these reports concerning fungal viruses have been based exclusively on electron microscopy. However, some fungal viruses have, in addition, been isolated and characterized biophysically, and, in a few instances, studies with fungal viruses have taken into consideration essentially all of Koch's postulates—namely, infection, transmission, curing, and reinfection.

The most extensively studied system is the mycovirus of *Penicillium chrysogenum* (12, 14, 28, 35, 47, 136-138, 157, 161, 167, 209, 215, 222),

the mold employed for commercial production of penicillin. Fungal viruses, however, were not initially discovered in *P. chrysogenum* but rather in another industrial fungus.

Discovery of Virus Particles in the Cultivated Mushroom

As early as 1950, a disease of the cultivated mushroom, *Agaricus bisporus*, was described by Sinden and Hauser (190). The symptoms of the disease were a marked decrease in production of mushrooms, the development of mushrooms with distorted morphology, and a premature deterioration of mushroom tissue. This disease, or at least a similar combination of symptoms, was reported subsequently by other investigators (79, 80, 111, 196). The transmissibility of the disease was studied extensively by Gandy (80–82), but it was Sinden (191) who first suggested that the disease might be attributable to a virus. These early studies on a disease of the commercial mushroom led to the first observation of a fungal virus by electron microscopy (83). Hollings and co-workers subsequently identified as many as three morphologically distinct viruses in association with diseased mushrooms (94, 95, 98, 101). Between 1962 and 1965, considerable evidence for viruses in *A. bisporus* accumulated. These viruses were partially purified through density-gradient centrifugation, and the infective and pathogenic nature of the viruses was confirmed. Purified viruses had an absorption profile characteristic of nucleoprotein, and immunological tests indicated that at least two of the viruses were serologically active and distinct.

Viral Double-Stranded Ribonucleic Acid in Species of Penicillium

Viruses were independently discovered in another group of fungi, the *Penicillium* molds. This discovery developed specifically from studies with two species, *Penicillium stoloniferum* and *Penicillium funiculosum*.

In the early 1950s, during a search for compounds effective against poliovirus, interest developed in pharmaceutical research over antiviral substances apparently synthesized by these two *Penicillium* species (170, 187). It is now known that the active antiviral substance associated with these two molds is double-stranded ribonucleic acid (dsRNA) of viral origin.

Progress in research on the antiviral activity associated with cultures of *Penicillium* was slowed during the late 1950s but was renewed with the observation that substances from these two molds were capable of inducing interferon in tissue culture and in laboratory animals (108, 179). Thus, a specific biological activity, interferon stimulation, made it possible to assay fractions derived from these molds for their antiviral activity. Kleinschmidt and Ellis (67, 109) demonstrated through electron microscopy that an active antiviral fraction from *P. stoloniferum* contained polyhedral virus particles. Lampson and co-workers simultaneously reported that the interferon inducer from *P. funiculosum* was dsRNA of presumed viral origin (120). Kleinschmidt and co-workers subsequently reported that virus particles isolated from *P. stoloniferum* in fact contained dsRNA. The viral nature of the dsRNA from both *P. stoloniferum* and *P. funiculosum* was confirmed by Banks and co-workers (15). Viruses containing dsRNA were soon discovered in other species of *Penicillium* (12, 100, 138, 156, 157, 218).

Early Reports of Viruses in Other Fungi

Prior to 1968, indicative evidence for viruses in fungi other than *A. bisporus* or the *Penicillium* species had been reported. These reports were based principally on descriptive plant pathology or on the anomalous segregation and transmission of certain genetic determinants. These studies, however, did not present convincing evidence for the presence and replication of viruses in the fungal cell. Nevertheless, these reports are reviewed here because of their historical importance to this subject.

Presumptive evidence for a virus in yeast was published as early as 1936 (212), and a lytic phenomenon in yeast, presumably associated with a viral infection, was described considerably later by Lindegren and co-workers (92, 145, 146). An ultrastructural examination of degenerative yeast cells revealed the presence of pleomorphic, electron-dense particles bounded by a double membrane (92). These particles were interpreted by Lindegren and co-workers to be viruses, but they were not isolated or characterized further, and their viral nature remains in doubt to this day.

Blattný and Pilát (22) described morphologically distorted forms of several species of mushrooms and attributed such distortions to viruses. Ellingboe (66), in an attempt to explain an abnormal recombination of genetic markers in *Schizophyllum commune*, suggested that this basidiomycete might harbor a virus capable of transducing specific genes. However, only circumstantial evidence for viruses among basidiomycetes was presented by these authors.

Cytoplasmic determinants for abnormal growth or for cellular breakdown have been reported for a number of fungi (19, 44, 50, 69,

103, 143, 144, 151, 155, 171). In some instances, such as in certain "petite" segregants of yeast or "poky" strains of *Neurospora*, these phenomena are determined by mitochondrial defects. In other instances, these phenomena are less well understood, but they are transmissible as cytoplasmic factors.

A transmissible disease of *Helminthosporium victoriae* was described by Lindberg in 1959 (143). Cellular extracts from abnormally stunted colonies of this fungus induced normal colonies to yield abnormal sectors. The infective agent was isolated from abnormal cells and concentrated by differential centrifugation and

TABLE 1. *Distribution of fungal viruses*[a]

Fungal species	Reference(s)
Basidiomycetes	
Agaricus bisporus	8, 55, 63, 83, 94, 96, 98, 100, 121, 160, 197
Boletus sp.	100
Coprinus lagopus	184
Hypholoma sp	26
Laccaria laccata	21
Lentinus edodes	157a
Polyporus sp.	26
Puccinia graminis	158, 175
Schizophyllum commune	112
Thanatephorus cucumeris (= *Rhizoctonia solani*)	26
Tilletiopsis sp.	26
Ustilago maydis	52, 216
Ascomycetes	
Daldinia sp.	26
Diplocarpon rosae	29
Hypoxylon sp.	26
Neurospora crassa	119, 131, 202
Gaeumannomyces graminis (= *Ophiobolus graminis*)	128, 177
Peziza ostracoderma (= *Plicaria fulva*)	55
Saccharomyces carlsbergensis	208
Saccharomyces cerevisiae	16, 17, 23, 118, 206
Saccharomycodes ludwigii	118
Deuteromycetes	
Alternaria tenuis	100
Arthrobotrys sp.	26
Aspergillus flavus	149, 217, 217a
Aspergillus foetidus	13, 174
Aspergillus glaucus	100
Aspergillus niger	13
Botrytis sp.	26
Candida tropicalis	118
Candida utilis	118
Cephalosporium chrysogenum (= *Cephalosporium acremonium*)	49, 135
Chromelosporium sp.	123
Chrysosporium sp.	26
Colletotrichum lindemuthianum	175
Fusarium moniliforme	26
Gliocladium sp.	26
Gliomastic sp.	26
Gonatobotrys sp.	193a
Helminthosporium maydis	31
Helminthosporium oryzae	194
Helminthosporium victoriae	26
Kloeckera sp.	26
Mycogone perniciosa	126, 127, 129
Paecilomyces sp.	26
Penicillium brevicompactum	102, 83a, 218
Penicillium chrysogenum	11, 28, 47, 48, 137, 138, 161, 209, 222
Penicillium citrinum	25
Penicillium claviforme	152
Penicillium cyaneofulvum	12
Penicillium funiculosum	15, 26, 120
Penicillium multicolor	26
Penicillium notatum	100
Penicillium stoloniferum	15, 36, 37, 67, 109, 110
Penicillium variable	25
Periconia cincinata	65
Piricularia oryzae	74, 221
Rhodotorula glutinus	118
Sclerotium cepivorum	122, 125, 193
Scopulariopsis sp.	26
Spicaria sp.	26
Stemphylium botryosum	99, 169
Trichothecium sp.	26
Verticillium sp.	26
Phycomycetes	
Aphelidium sp.	182
Choanephora sp.	26
Mucor sp.	26
Paramoebidium arcuatum	150
Plasmodiophora brassicae	5
Rhizopus sp.	26
Schizochytrium aggregatum	106
Syncephalastrum sp.	26
Thraustochytrium sp.	106, 107
Myxomycetes	
Labyrinthomyxa marina (= *Dermocystidium marinum*)	168

[a] Included in this list are reports for which there is only minimal or electron microscope evidence for virus. In some of these reports investigators have been cautious to avoid use of the term "virus" or have designated particles as "virus-like." We have admittedly been presumptive in designating certain entries in the list as viruses, but we feel that nothing is to be gained by continued use of the term "virus-like particles."

precipitation with ammonium sulfate. Although Lindberg postulated that this transmissible disease might involve a virus, the activity was not filterable across a standard microbiological filter, and the active cellular extracts were not examined for virus by electron microscopy.

Transmission of a lethal cytoplasmic factor in *Aspergillus glaucus* was studied by Jinks (103). The factor was transmissible through heterokaryosis. From a specific cross involving a white-spored, afflicted strain and a buff-spored, healthy strain, an afflicted heterokaryon was obtained and analyzed. Less than 1% of the spores isolated from this heterokaryon germinated. However, from the surviving fraction of spores, heterokaryotic colonies as well as homokaryotic colonies with either white or buff spores were obtained. All three types of colonies exhibited lethal sectors indicative of transmission of some cytoplasmic determinant. Jinks considered this phenomenon to be mutational rather than viral in nature.

Several fungi have been implicated as vectors for the transmission of viral diseases to higher plants (85, 99, 200). *Olpidium brassicae* was reported in 1958 to transmit a virus responsible for big vein disease of lettuce (40, 77, 86), and subsequently this fungus was suggested as a possible vector for at least two viral diseases of tobacco plants (91, 93, 198, 199). Similarly, the fungi *Polymyxa graminis* (72) and *Synchytrium endobioticum* (164) have been postulated as agents for the transmission of viruses to plants. However, these reports, which are based largely on descriptive phytopathology, did not demonstrate that the viruses under consideration could replicate or were even present within the fungal cell.

In view of the wide and common distribution of viruses now evident among fungi, certain of the early presumptive evidence for viruses in fungi may indeed have had some basis in fact. Viruses particles have now been reported in strains of *S. commune* (112) and *A. glaucus* (100), and recent experimental evidence supports the view that viruses can infect yeast cells (16, 17, 23, 46, 117) and are present in various plant pathogenic fungi, including strains of *Helminthosporium* (26).

Recent and more detailed study on transmission of tobacco necrosis virus by *O. brassicae* indicates that this virus is merely adsorbed to the exterior of fungal cells (zoospores) (200). Evidence has likewise been presented that cucumber necrosis virus can adhere to the outer surface of *Olpidium* zoospores (54, 200). Dias (54), however, has reported that cucumber necrosis virus may occur within resting spores of *Olpidium cucurbitacearum*, but there is no evidence that this virus replicates in situ. Viruses associated with species of *Olpidium* can not at this time be classified as fungal viruses.

STRUCTURE AND BIOCHEMISTRY OF FUNGAL VIRUSES

Although virus particles have been observed in numerous fungal species, only a few fungal viruses have been described in detail. The biophysical properties of the most extensively studied fungal viruses are summarized in Table 2. Thus far, those fungal viruses characterized biophysically are remarkably similar. They are all small polyhedral or spherical particles with diameters of 33 to 41 nm, and they contain dsRNA. The hexagonal shape of negatively-stained particles suggests icosahedral symmetry (161, 174, 221; Fig. 1 and 2A). Fungal viruses containing dsRNA may be related to other dsRNA-containing viruses (Family: *Reoviridae* [148]), but formal classification of fungal viruses must await further study. The name *Mycorna* has been proposed for icosahedral mycoviruses containing dsRNA (123). Wood (214) has written a comprehensive review of viruses containing dsRNA.

Biophysical Characterization of Fungal Viruses

Examination of virus particles for electrophoretic mobility, sedimentation coefficient, buoyant density, and serological specificity reveals differences among those viruses infecting the *Penicillium* species, *Aspergillus* species, *Periconia circinata*, and *Ustilago maydis* (Table 2). *P. stoloniferum* and *Aspergillus foetidus* each contain two electrophoretically distinct viral components (30, 174) designated herein as *Ps*-1s, *Ps*-1f, and *Afo*-1s, *Afo*-1f, respectively. These virus particles, isolated by electrophoresis, can be delimited further by density gradient and equilibrium centrifugation into several virus-containing bands. The electrophoretically fast and slow viruses are antigenically distinct as demonstrated by formation of two immunoprecipitin lines on Ouchterlony double-diffusion plates. Virus populations from *P. stoloniferum* can also be resolved into two precipitin lines by immunoelectrophoresis (36). Thus, *A. foetidus* and *P. stoloniferum* each harbor at least two serologically distinct viruses.

Virus particles isolated from *Aspergillus niger* are serologically related to *Afo*-1s and *Afo*-1f from *A. foetidus* and possess similar biophysical properties (174). Particles from *Diplocarpon*

rosae are related serologically to the *Ps*-1s of *P. stoloniferum* but do not cross-react with *Ps*-1f (29).

Multiple bands containing virus have been reported in sedimentation profiles of virus preparations from *Ustilago maydis* (216), *Periconia circinata* (65), *Penicillium stoloniferum* (37), *Penicillium brevicompactum* (218), and *Penicillium chrysogenum* (215). Viruses which infect the latter two fungi are serologically related and have similar biophysical properties. One minor peak, 218*S*, observed in sucrose density gradients of the *P. chrysogenum* and *P. brevicompactum* viruses has been attributed to formation of dimers (215, 218). It is also possible that several sedimenting components of one virus could result if aggregation occurred between "full" and "empty" particles. Several investigators have observed empty viral particles in electron micrographs (37, 161, 217, 218, 221; Fig. 1 and 2A), but this apparent emptiness could represent an artifact of staining, i.e., penetration of a damaged virion by negative stain.

Buck and Kempson-Jones (37) have determined that the electrophoretically slow virus of *P. stoloniferum* (*Ps*-1s) is in fact heterogeneous for at least seven types of particles. Although particles are all of the same dimension, 34 nm in diameter, differences in sedimentation and buoyant density are observed (Table 2), and these differences can be explained by variation among particles for nucleic acid content (see below for details). One particle type, *Ps*-1sE, lacks nucleic acid and is indeed empty. Virus particles devoid of nucleic acid are apparently also found in *Aspergillus flavus* (217, 217a).

Two different values have been reported for

TABLE 2. *Biophysical properties of fungal viruses*

Fungus (virus[a])	Morphology	Diam (nm)	Electrophoretic component	Sedimentation		Immunodiffusion	Reference(s)
				Sedimentation coefficient[b]	Buoyant density (g/cm³)		
Penicillium chrysogenum (*Pc*-1)	Polyhedral	35	Single[c]	150	1.27 (K tartrate)	Single	35
	Polyhedral	35			1.38 (CsCl)	Single	138, 161
	Polyhedral	40	Single[d]	150 major 218 minor	1.354 (CsCl) Several minor bands	Single	215
P. brevicompactum (*Pb*-1)	Polyhedral	36-40	Single[d]	147 major 128 minor		Single	218
P. stoloniferum							
(*Ps*-1f)	Polyhedral	34	Fast[d]	Several	Several components	Two	27, 30, 36, 203
(*Ps*-1s)	Polyhedral	34	Slow[d]	Several	1.299-1.376 (CsCl)		
(*Ps*-1sE)	Polyhedral	34		66	1.297		37
(*Ps*-1sM1)	Polyhedral	34		87	1.332		37
(*Ps*-1sM2)	Polyhedral	34					37
(*Ps*-1sL1)	Polyhedral	34		101	1.358		37
(*Ps*-1sL2)	Polyhedral	34			1.362		37
(*Ps*-1sH1)	Polyhedral	34		113	1.384		37
(*Ps*-1sH2)	Polyhedral	34			1.390		37
P. cyaneofulvum (*Pcy*-1)	Polyhedral	32.5		157	1.39 (CsCl)	Single	12
Ustilago maydis (*Um*-1)	Spherical	41		Five components; 110-160		Single	216
Periconia circinata (*Pci*-1)	Polyhedral	32		Three major components; 66, 140, 150			65
Aspergillus foetidus							
(*Afo*-1s)	Polyhedral	33-37	Slow[e]	Two components; 146-172	Four components; 1.396-1.435 (CsCl)	Two	13, 174
(*Afo*-1s)	Polyhedral	33-37	Fast[e]	Four components; 145-158	Four components; 1.351-1.380 (CsCl)		

[a] Fungal viruses are designated here and elsewhere in the text by initials.
[b] Sedimentation values derived from either analytical centrifugation or sucrose density-gradient centrifugation.
[c] Polyacrylamide electrophoresis.
[d] Sucrose-density gradient electrophoresis.
[e] Agarose electrophoresis.

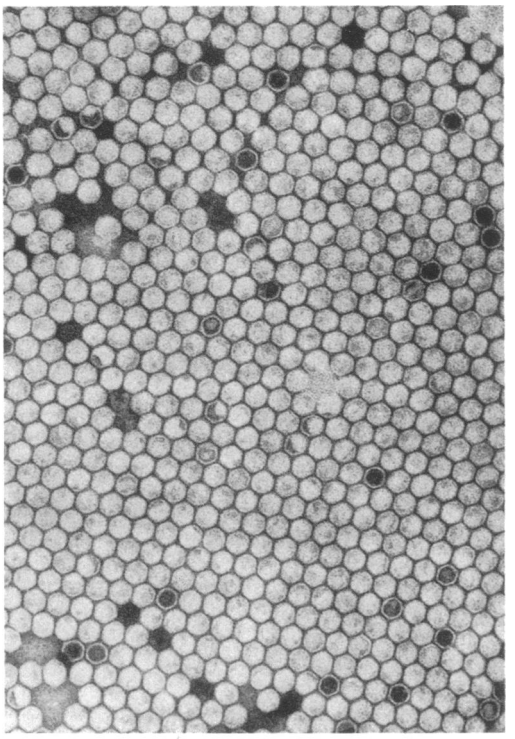


FIG. 1. *Virus particles purified from Piricularia oryzae and stained with 2% potassium phosphotungstate (×100,000). (Micrograph courtesy of S. Yamashita, Y. Doi and K. Yora [221].)*

the buoyant density of the *P. chrysogenum* virus in CsCl, 1.38 and 1.354 (161, 215). In addition, Wood and Bozarth (215) have reported several minor viral components in CsCl gradients which were not observed by Nash and co-workers (161) in CsCl or by Buck and co-workers (35) in potassium tartrate. The buoyant density of viruses may be altered by the age of the virus, the duration of centrifugation, and the pH of the gradient. *P. chrysogenum* virus appears to be unusually susceptible to disruption by freezing and thawing (161). This fragility could also contribute to multiple sedimenting components.

The viruses from *P. chrysogenum* and *P. brevicompactum* each form one precipitin band on Ouchterlony double-diffusion tests. Moreover, particle preparations from *P. chrysogenum* and *P. brevicompactum* contain a single electrophoretic component.

The molecular weight of the *P. chrysogenum* virus has been calculated from its sedimentation rate, $s_{20,w} = 145S$, its diffusion coefficient, $D_{20w} = 1.03 \times 10^{-7}$ cm²/s, and its partial specific volume to be 13.0×10^6 (215). Chemical analysis of purified *P. chrysogenum* and the closely related *P. brevicompactum* viruses indicates that 85 to 90% is protein and 11 to 15% is RNA (215, 218). The molecular weight of L-type particles from the electrophoretically slow virus of *P. stoloniferum*, *Ps*-1sL, has been calculated from sedimentation and diffusion coefficients to be 6.0×10^6 (37). These particles are calculated to be approximately 15% RNA. The H-type particles of this virus, however, have a composition of 25% RNA, M-type particles are composed of only 9% RNA, and E-type particles lack nucleic acid (37).

Amino acid analysis has been carried out on purified virus from *P. chrysogenum*. The amino acids present are those normally associated with protein (Nash and Lemke, unpublished results; Table 3).

Double-Stranded RNA Genome

The nucleic acids extracted from viruses infecting *P. chrysogenum* (35, 161), *P. brevicompactum* (218), *P. funiculosum* (15), *P. stoloniferum* (15, 110), *Periconia circinata* (65), *U. maydis* (216), *A. niger* (13), and *A. foetidus* (13, 174) have proven to be RNAs. Recently, Velikodvorskaya and co-workers (205) described two DNA-containing bacteriophage-type viruses from *P. brevicompactum*, and Kazama and Schornstein (106) have reported a herpes-type virus from a *Thraustochytrium* sp. The deoxyribonucleic acids assumed to be present in these viruses, however, have not been characterized, and the two bacteriophage-type viruses reported in the Russian experiments (205) may indeed have been derived from bacterial contaminants.

Cellular inclusions containing single-stranded RNA have been isolated from a mutant strain of *Neurospora crassa* (119). These particles are polymorphic and 250 to 400 nm in diameter, and individual particles are defined by a unit membrane. Superficially, these particles resemble the virus-like particles described from yeast by Lindegren and co-workers (92, 145). The particles from *N. crassa* are associated with a mitochondrial fraction from a specific respiratory-deficient mutant, *abnormal-1*. These particles, if indeed viral in nature, are clearly unlike any of the viruses characterized thus far from fungi.

Biophysical properties of the more extensively characterized viral nucleic acids from fungi are shown in Table 4. Purified nucleic acids from these fungal viruses have a typical ultraviolet absorption spectrum for nucleic acid and give a positive orcinol reaction and a negative diphenylamine reaction. Hydrolysis of

purified viral nucleic acids from *P. chrysogenum* and *P. stoloniferum* yields evidence for four bases including uracil (42, 38, 147). These viral nucleic acids are not degraded by deoxyribonuclease (DNase) treatment and, in general, are sensitive to ribonuclease (RNase)

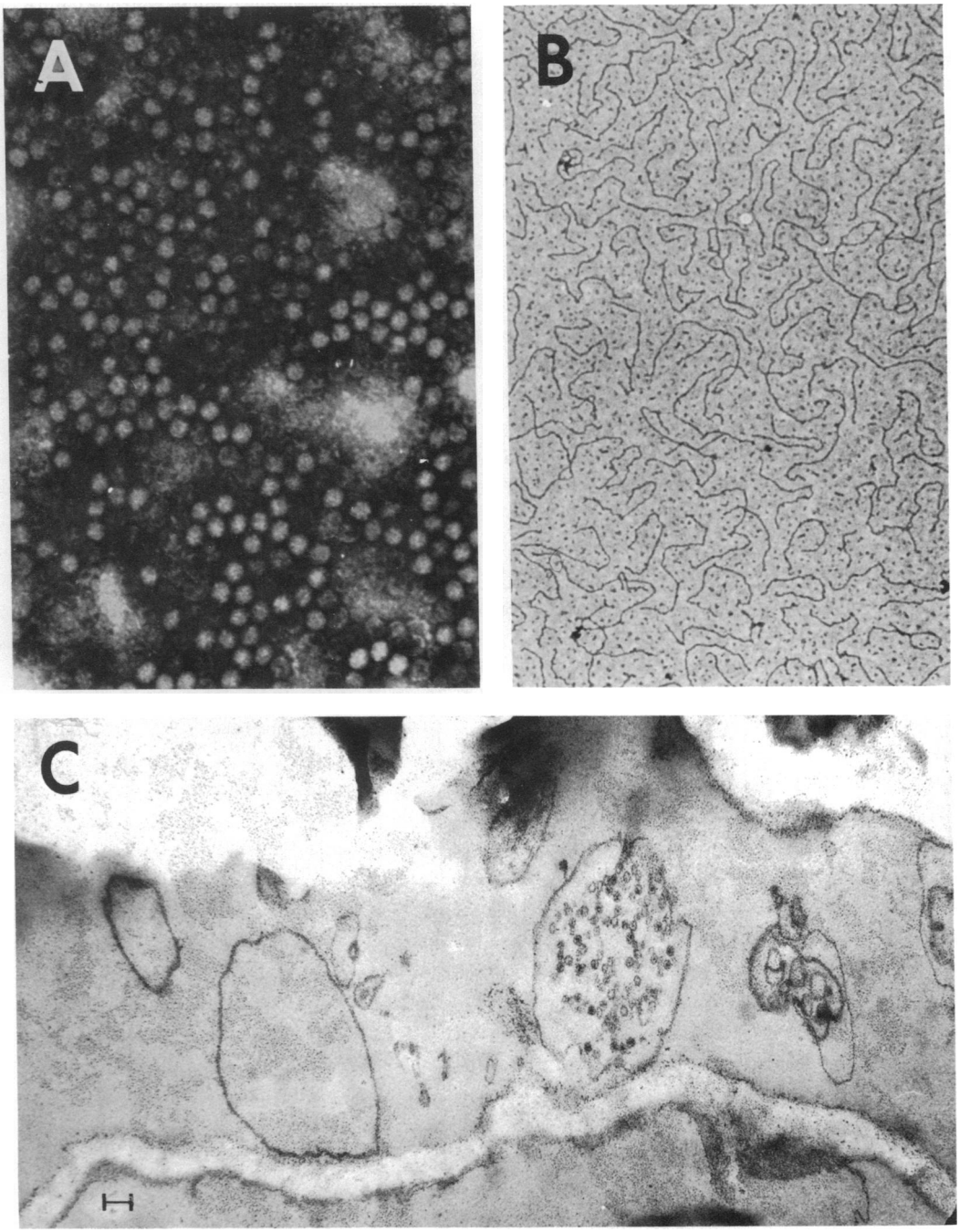


FIG. 2. *A, Virus particles purified from Penicillium chrysogenum (×100,000). B, Molecules of dsRNA derived from purified virus of P. chrysogenum and stained with 0.05 M uranyl acetate (×30,000). C, Thin-section of a cell of P. chrysogenum showing a vesicle filled with virus particles (inset scale, 100 nm). (Micrograph 2C courtesy of O. Volkoff, T. Walters, and R. Dejardin [209].)*

TABLE 3. *Amino acid composition of purified virus from Penicillium chrysogenum*[a]

Amino acid	μmol/unit (OD_{260}) of virus
Aspartic acid	0.197
Glutamic acid	0.192
Leucine	0.190
Alanine	0.164
Glycine	0.148
Serine	0.128
Threonine	0.122
Valine	0.122
Arginine	0.114
Lysine	0.098
Methionine	0.087
Proline	0.085
Isoleucine	0.084
Phenylalanine	0.068
Tyrosine	0.068
Histidine	0.040
Cysteine	Trace

[a] Amino acid composition was determined with a Beckman amino acid analyzer according to the procedure of Hamilton (87).

only at low ionic strengths. These data are all indicative of dsRNA.

The dsRNA extracted from purified *P. chrysogenum* virus has a mean contour length of 0.86 μm which corresponds to a molecular weight of 2.0×10^6 (161; Fig. 2B). This viral RNA has a buoyant density of 1.60 to 1.61 g/cm^3 in Cs_2SO_4 gradients which corresponds to that expected for dsRNA. A minor single-stranded RNA (ssRNA) which bands at a density of 1.69 g/cm^3 has been reported in *P. chrysogenum* and *A. foetidus* viral nucleic acid preparations (161, 174). This single-stranded material has not been further characterized, and its function is unknown. ssRNA components have also been found among virus particles of *P. stoloniferum* and have approximately one-half the molecular weight of the dsRNA (30, 37).

Sedimentation coefficients for dsRNA from *P. chrysogenum*, *P. brevicompactum*, and *A. foetidus* range from 12.6 to 13.5, which corresponds to molecular weights from 1.6×10^6 to 2.0×10^6.

Purified viral RNA can often be resolved into

TABLE 4. *Biophysical properties of nucleic acids from fungal viruses*

Fungus (virus)	Resistance[a] to RNase	Thermal melting (T_m; C)	Sedimentation coefficient ($s_{20,w}$)	Buoyant density (g/cm^3)	Polyacrylamide electrophoresis (mol wt × 10^6)	Reference(s)
Penicillium chrysogenum (*Pc*-1)	+	86 (0.1 SSC) 84 (0.01 SSC)	13 13	1.61 (Cs_2SO_4) 1.60 (Cs_2SO_4)	Three bands 2.18, 1.99, 1.89	35, 161, 215
P. brevicompactum (*Pb*-1)	+	84 (0.01 SSC)			2.18, 1.99, 1.89	218
P. stoloniferum						
(*Ps*-1f)	+		10–12		0.99, 0.89, 0.24	30, 36
(*Ps*-1s)	+				1.10, 0.94	
(*Ps*-1sE)					No nucleic acid	37
(*Ps*-1sM1)	−	63 (SSC)	16.4		0.47 (ssRNA)	37
(*Ps*-1sM2)	−				0.56 (ssRNA)	37
(*Ps*-1sL1)	+	102 (SSC)	11.7		0.94	38
(*Ps*-1sL2)	+				1.11	37
(*Ps*-1sH1)	±	63 + 102 (SSC)	11.7, 15.3		0.94 + ss component[b]	37
(*Ps*-1sH2)	±				1.11 + ss component	37
P. cyaneofulvum (*Pcy*-1)	+	101 (SSC)	12.5			12
Ustilago maydis (*Um*-1)	+	80 (0.01 SSC)			2.87, 2.52, 0.93 0.49, 0.44, 0.06	216
Periconia circinata (*Pci*-1)	+		11.5, 13.5		1.75, 1.40, 1.25 1.10, 0.48, 0.42	65
Aspergillus foetidus						
(*Afo*-1s)	+	100 (SSC)	13.5		2.24, 2.76	159
(*Afo*-1f)	+	88 (0.1 SSC)	13.5		1.44, 1.70 1.87, 2.31	

[a] Resistance at ionic strength of standard saline citrate (SSC = 0.15 M sodium chloride plus 0.015 M sodium citrate, pH 7.2).
[b] ss component means single-stranded RNA component.

multiple components by polyacrylamide gel electrophoresis. Molecular weights of these components can be determined by using reovirus dsRNA as a standard. Each of the two viruses harbored by *P. stoloniferum* and *A. foetidus* contain two to four closely related dsRNA molecules.

Buck and Kempson-Jones (37) have shown that ssRNA as well as dsRNA molecules are distributed among at least six component types of particles in the electrophoretically slow virus of *P. stoloniferum* (Table 4). Ratti and Buck (174) found that the six classes of dsRNA from *A. foetidus* virus particles had separate densities in CsCl gradients. The three electrophoretic components of dsRNA from the *P. chrysogenum* virus, however, are not resolved in CsCl gradients (161). Electron microscopy of partially degraded *P. chrysogenum* virus particles indicates that each particle contains only a single molecule of dsRNA of about two million molecular weight (215). The component forms of dsRNA in *P. chrysogenum* virus could represent differences in either molecular weight or conformation (161). Multiple RNA components have been reported in RNA viruses of animals (9, 185, 189) and higher plants (78, 204). If the *P. chrysogenum* virus in fact possesses a segmented genome, then each dsRNA component must be encapsulated into separate particles (215). Wood (214) in a general review of viruses with dsRNA genomes has emphasized that, among the dsRNA-containing viruses, the genomes of the mycoviruses are characteristically small and apparently unusual in that their genomic segments may be encapsulated in individual particles.

The secondary structure of viral dsRNA isolated from *P. chrysogenum* has been studied by circular dichroism, thermal denaturation, and binding of ethidium bromide. The circular dichroic spectrum of this RNA is typical of a double-stranded nucleic acid (47, 161). Nash and co-workers (161) reported that thermal melting of dsRNA was a linear function of the negative logarithm of the sodium ion concentration. These data indicate a double-helical structure for the viral RNA rather than order-disorder transitions involving single strands. Ethidium bromide intercalates with RNA from *P. chrysogenum* virus in a manner similar to dsDNA, which further suggests a double-helical structure for the nucleic acid of *Pc*-1 virus (64).

REPLICATION OF FUNGAL VIRUSES

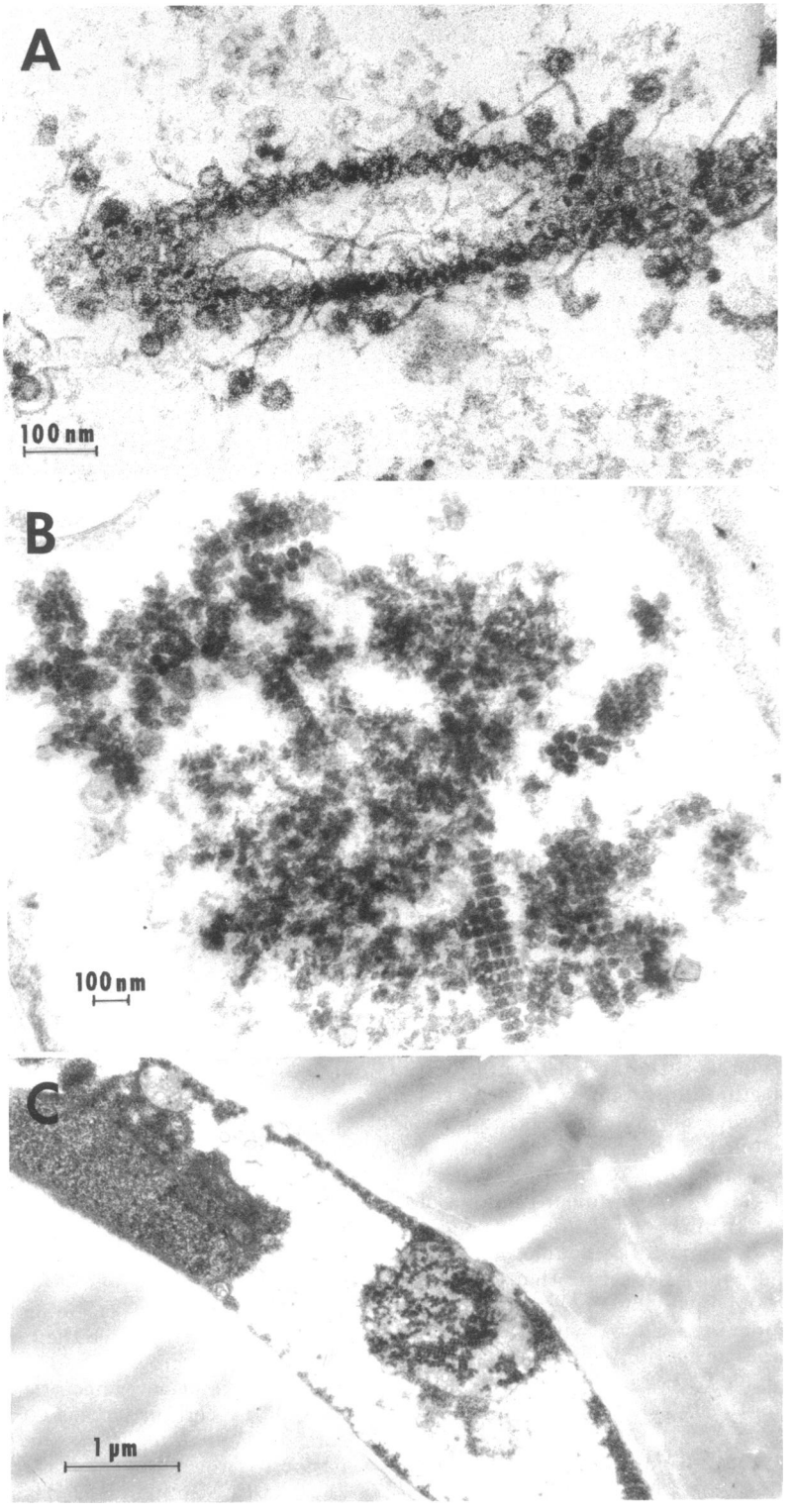
The replication of fungal viruses has not been extensively studied due to the difficulty of measuring viral titers and to the cellular complexity of the eukaryotic fungal host. Growth in filamentous fungi proceeds through apical or tip elongation of hyphae, and the viruses present in fungi are generally latent. Fungal cell lines infected with virus mature without regular or predictable lysis.

Ultrastructural Aspects of Virus Replication in the Fungal Cell

Electron microscope studies suggest an increase in titer and organization of fungal viruses with aging of the hypha. Border et al. (24) examined the distribution of virus particles in thin sections of hyphae from several species of *Penicillium*. Young apical regions of hyphae were free of virus particles, whereas older regions contained many particles. Viruses in older cells appeared to aggregate into extensive crystalline arrays and ultimately become enclosed in vesicles. Aggregation of virus particles and association of aggregates with membranes have been observed in thin sections of hyphae from *P. chrysogenum* (209, 222; Fig. 2C), *Penicillium cyaneofulvum* (24). *P. funiculosum* (24), *P. brevicompactum* (102), *A. bisporus* (6, 58, 60; Fig. 3), *P. stoloniferum* (1, 45, 102; Fig. 4A), *Peziza ostracoderma* (62), *Saccharomyces cerevisiae* (23), *Thraustochytrium* sp. (106, 107), and *A. foetidus* (14). The aggregation of viruses in these fungi resembles that observed in other eukaryotic cells. Herpes virus (165, 186), adenovirus (220), and certain plant viruses (210) aggregate within cells of their respective hosts and may be associated with membranous structures.

Metitiri and Zachariah (152) examined the organization of virus particles in a culture of *Penicillium claviforme* grown in liquid medium. In the early stages of growth, small aggregates of particles as well as crystalline protein bodies were evident in cells. Subsequently, particles appeared at high titer but were scattered throughout the cytoplasm.

Viruses are released into liquid medium by strains of *P. stoloniferum* (99) and *P. chrysogenum* (137). The release of viruses into liquid medium is prevalent in older cultures and is probably a consequence of autolysis. Autolysis in *Penicillium* is promoted by cultural conditions which reduce the pH of the medium (135). Specific growth conditions are known to enhance formation of necrotic lesions in plants infected by certain viruses (10, 201, 211). Cultures of *Penicillium citrinium* and *Penicillium variabile* grown on solid medium containing 18% lactose exhibit localized lysis (25). The lysis is restricted to patches of nonsporulating hyphae. These hyphae, prior to lysis, contain high


A
100 nm
B
100 nm
C
1 µm

titers of virus in a generally disorganized cytoplasm.

Infection by viruses persists rather indefinitely in some fungi. Strains, once infected, remain infected after several transfers (57, 102, 137). Cultures derived as single spore isolates from infected species of *Penicillium* and *Aspergillus* are routinely infected with virus. Strains of *P. chrysogenum* developed for the industrial production of penicillin have been derived from one culture (NRRL-1951) through an extensive series of mutations (183). Each mutant has retained infection by *Pc*-1 virus, as strains used currently for production of penicillin are consistently infected (11, 137).

Viruses have been observed in thin sections of fungal spores (45, 58, 60, 102; Fig. 4B). Ultrastructural examinations of spores from infected strains of *P. stoloniferum* (102) and *A. bisporus* (58, 59) indicate that virus particles are present in exceedingly higher titer in spores of these fungi. Fungal viruses harbored by *A. bisporus* are thus readily transmitted through basidiospores (59, 99, 181). However, ascospores derived from virus-infected strains of *Gaeumannomyces* (*Ophiobolus*) *graminis* apparently do not incorporate viruses (99, 128, 181). Virus particles and viral dsRNA have been isolated from spores (conidia) of *Penicillium* species (180). The viral titer in conidia of these fungi proved to be quite comparable to that found in mycelial preparations (180). The presence of fungal viruses in spores would permit viruses to be stored and to be transported during nonvegetative phases of the fungal life cycle.

RNA Polymerase Activity Associated with Viruses of Penicillium

The genetic events in the replicative cycle of fungal viruses remain a mystery. Large quantities of free, heterogeneous dsRNA have been isolated from species of *Penicillium* (14, 47, 120, 138) and *Aspergillus* (159). This nucleic acid probably represents a pool of uncoated replicative intermediate involved in the synthesis of virus. The crystalline protein bodies observed in *P. claviforme* may be unassembled capsid protein (152).

Recently, RNA polymerase activity was reported with purified virus preparations from *P. stoloniferum* (124) and *P. chrysogenum* (136, 161). Lapierre and co-workers (124) observed that the enzyme activity from *Ps*-1 virus was not affected by actinomycin D, rifampin, or DNase but was sensitive to ethidium bromide. Ethidium bromide is known to intercalate with dsRNA from *Pc*-1 virus (64). Further experimentation, however, is needed to determine the template specificity and the nature of the product of the polymerases from *Penicillium* viruses. The presence of RNA polymerase in these viruses suggests that the dsRNA-containing viruses of fungi possess the capacity to replicate in the host cell in a manner analogous to replication of RNA-containing viruses in other organisms (9, 185).

Kaempfer and Kaufman (105) have recently reported that the dsRNA from *P. chrysogenum* virus is a potent inhibitor of protein synthesis in mammalian systems. The dsRNA binds with an initiation factor required for recycling of ribosomes into polyribosomes. In view of this finding, it now seems improbable that replication of dsRNA-containing mycovirus can occur with impunity to the fungal cell. It is not now known if dsRNA inhibits polyribosome formation in fungi.

VIRULENCE OF FUNGAL VIRUSES

There is at present no evidence to indicate that fungal viruses engage in genetic transformation of their host. Genetic recombination via transduction and transformation is, of course, characteristic of bacteria and their viruses (89), and there is no reason, a priori, to rule out the possibility that viruses in fungi also engage in genetic determination beyond their replication. At this time, however, any discussion regarding virulence of fungal viruses must proceed without consideration of such genetic details.

Fungal viruses, nevertheless, exhibit degrees of virulence ranging from apparent latency to overt lysis. Virulence varies even among strains of a given species. In general, fungal viruses appear to be relatively avirulent or latent, since apparently healthy mycelia often contain high titers of virus particles (14, 59, 161, 222). Growing cells of *P. chrysogenum* and *P. stoloniferum*, for example, may contain viral titers in the order of 1 mg of virus per g (dry weight) of cells (14, 161).

In some instances, however, mycelia infected with virus are obviously diseased. Infected mycelia of *A. bisporus*, for example, grow slowly and are severely impaired for the production of mushrooms (56, 83, 99). Lytic phenomena,

FIG. 3. *Virus particles in cells of Agaricus bisporus. A, Thin section of cell from stipe of mushroom showing virus particles in a linear array (insert scale, 100 nm). B, Virus particles in crystalline arrays in growing cell of A. bisporus (inset scale, 100 nm). C, Vesicle containing virus particles in hypha of A. bisporus (insert scale, 1 μm). (Unpublished micrographs courtesy of T. Walters and O. Volkoff, University of British Columbia.)*

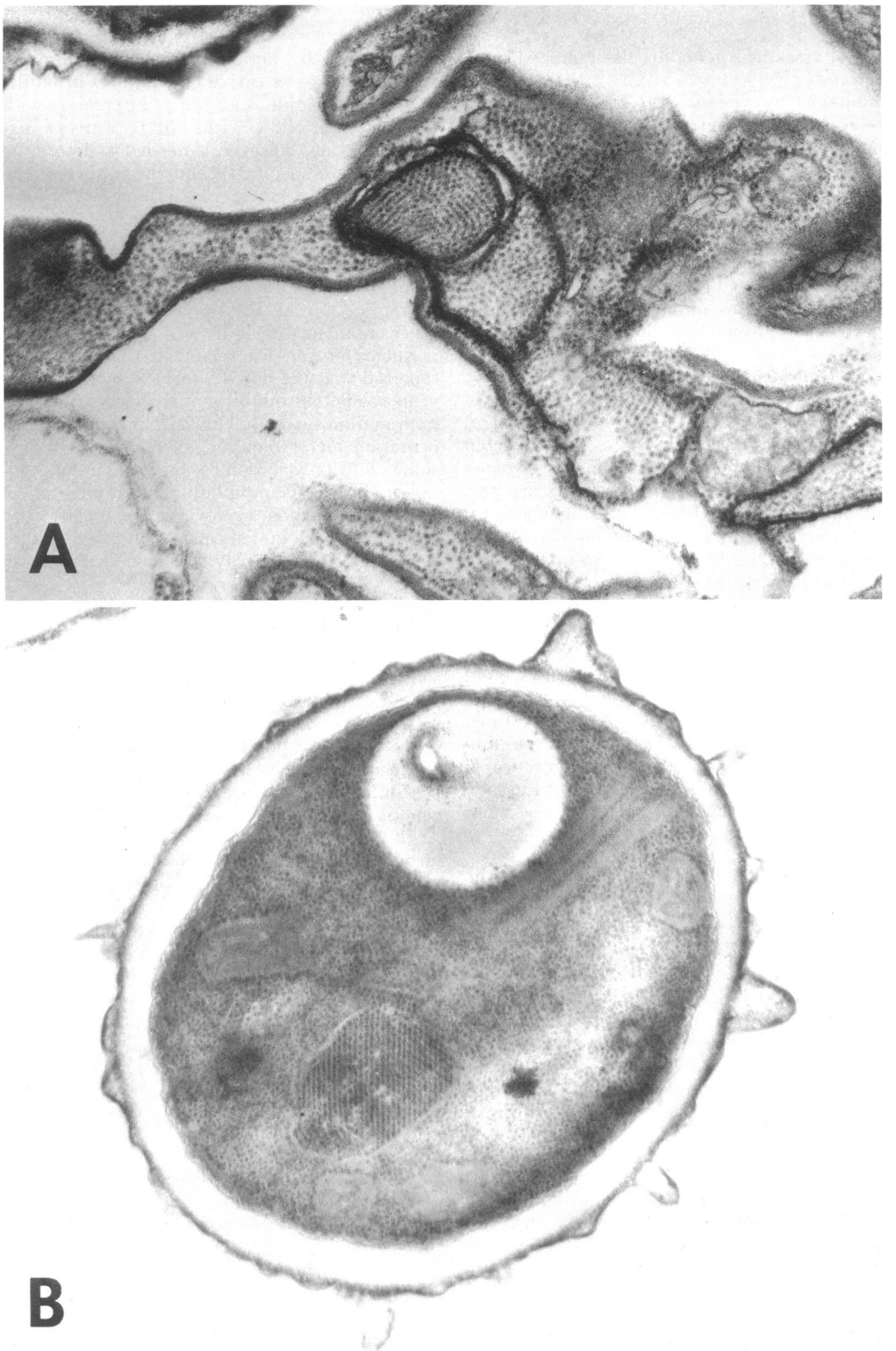


FIG. 4. *A, Virus particles present in vesicles or scattered throughout cells of Penicillium stoloniferum (×38,000). B, Thin section of spore of P. stoloniferum containing virus particles in crystalline arrays and scattered throughout the cytoplasm (×39,000). (Unpublished micrographs courtesy of M. Corbett, University of Maryland.)*

moreover, occur in some fungal cell lines infected with viruses. Lytic plaque formation occurs in some strains of at least three species of *Penicillium* (25, 137), and in some strains of *S. commune* (112). Degeneration of mycelia and lethality occurs irregularly in several other fungi, and genetic systems which control these phenomena often involve cytoplasmic determinants (17, 19, 42, 44, 50, 103, 143, 151, 162, 171). The "killer" phenomena of *U. maydis* and *S. cerevisiae* are two cases in point. Recent evidence indicates that these phenomena are somehow influenced by the presence of virus (16, 51, 52, 76, 206, 216).

Fungal viruses undoubtedly influence host metabolism, at least insofar as any virus, in order to multiply, must compete with its host for synthesis of protein and nucleic acid. As already suggested, the dsRNA viruses of fungi may impose a special burden upon their host for synthesis of protein.

Many fungi, in addition to their basic metabolism, synthesize unusual metabolites. Synthesis of these secondary metabolites may also be influenced by the presence of a virus. The genetic basis for the biosynthesis of many fungal metabolites is virtually unexplored.

Several fungi are pathogenic to other organisms, and the pathogenicity of these fungi may in certain instances be influenced by virus. Fungi have been repeatedly implicated as vectors for the transmission of viral diseases to higher plants (85, 99).

The biological consequences of viruses in fungi appear to be many and varied but can be discussed, at this time, with reference to only a few fungal systems.

Disease Symptoms of Virus in the Cultivated Mushroom

To date, the most destructive of fungal viruses described are found in *A. bisporus*. Viral disease symptoms in this cultivated mushroom are highly variable, and at least three types of pathogenic viruses occur in the fungus (59, 96; Fig. 5). These viral diseases are spread readily by either spores or mycelia infected with viruses (59, 99, 181), and no strain of *A. bisporus* is known to be resistant to virus. The most common symptoms of a viral disease in *A. bisporus* are sparse production of mushrooms and certain characteristic structural abnormalities in the mushrooms that are produced (Fig. 6). Although these symptoms are indicative of virus, similar symptoms can develop either under poor growing conditions or from infection of mushrooms by certain nonviral pathogens (189). Therefore, the viral nature of a disease in *A. bisporus* is determined only by isolation and identification of virus particles from suspect mushrooms (Fig. 5). Virus particles apparently are not found in tissue or spores from healthy mushrooms (58, 60, 61).

Dielman-van Zaayen (59) has attempted to obtain virus-free strains of *A. bisporus* by heat treatment of infected cultures. The fungus is mesophilic, as temperatures over 30 C are restrictive for growth, and 35 C is lethal. Among strains recovered after treatment with heat (33 C) were strains that exhibited increased productivity for mushrooms and contained reduced virus titer. However, no strain was freed of virus, and remission in virus titer and disease symptoms was only temporary. Recently, Hollings has reported that virus-free strains of *A. bisporus* can be obtained through treatment with heat, provided hyphal tips are isolated both before and after heat treatment (97). Nair (160a) has contended that heat therapy is effective if young mycelia are treated for at least 3 weeks at 33 C. Nair's studies have also indicated that growth rate is not a reliable criterion whereby to judge virus infection in *A. bisporus*.

Viral Plaque Formation in Species of Penicillium

Viruses containing dsRNA are present in many species of *Penicillium* and *Aspergillus* (Table 4). In three species of *Penicillium*, viruses are sometimes associated with lytic plaque formation (25, 137). The virus of *P. chrysogenum*, *Pc*-1, has been studied most extensively in this regard (Fig. 7, 137). Plaques are not routinely formed by all strains of this fungus known to contain virus. Indeed, many strains of *P. chrysogenum* with a high titer of virus (i.e., 1 mg of virus per g [dry weight] of cells) do not form plaques. However, regular plaque formation occurs in some strains, and this can be correlated with an increase in extracellular virus titer. Variation in viral plaque formation in *P. chrysogenum* may be related to altered sensitivity of the host to virus. Nuclear genes for resistance to lysis have been identified in strains of other fungi (19, 51, 103, 112, 192).

Strains of *P. chrysogenum* have been investigated for reduction in virus titer after treatment with either heat (74 C) or specific antimetabolites (137). Some strains treated with either heat or cycloheximide were greatly reduced for virus titer, and this reduction was demonstrated by an immunodiffusion assay (Fig. 8). One strain was apparently freed of virus since assay for virus by an extremely sensitive radioimmunological test was negative. The strain cured of virus exhibited an increased growth rate and

stability. This strain, moreover, lost the ability to form plaques. This strain was also used in reinfection experiments designed to determine if virus infection and plaque formation in *P. chrysogenum* were related (see below).

Genetic Resistance to Lysis in Schizophyllum commune

Lytic plaque formation occurs in some strains of *S. commune*, a sexually reproducing fungus with multiple mating types (112). This fungus is more closely related to *A. bisporus* than to any of the *Penicillium* species, and has been studied extensively as a genetic system (113, 173).

Virus-like particles in *S. commune* are spherical, averaging 130 nm in diameter. These particles are prevalent in cells from plaqued regions of a culture. The virus, *Sc*-1, is present in relatively low titer and has not been purified.

Plaque formation in *S. commune* is transmissible through heterokaryosis, and the inheri-

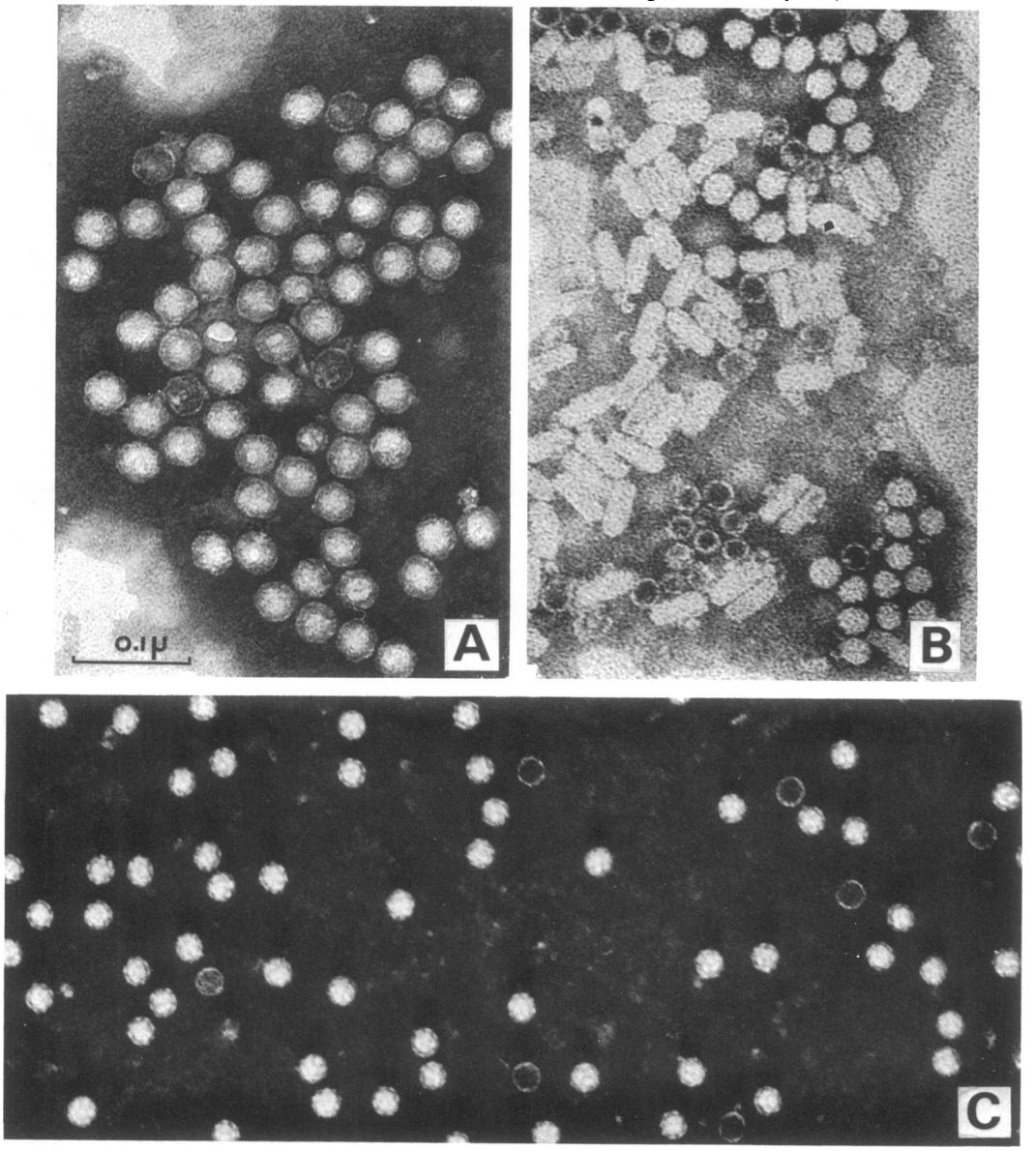


FIG. 5. *Virus particles isolated from diseased mushrooms of Agaricus bisporus. A, Particles 34 nm in diameter are hexagonal in outline; some 25-nm particles are also present. B, Bacilliform particles, 19-nm wide and 50-nm long; some 25-nm particles are also present. C. Isometric particles 25 nm in diameter. (Micrographs are of the same magnification; courtesy of A. Dieleman-van Zaayen and J. H. M. Temmink [63].)*

tance of plaque formation is cytoplasmic (112). In a specific cross involving a plaque-forming strain and a normal strain, almost 90% of progeny were plaqued.

Not all strains of *S. commune*, however, are sensitive to the presence of virus. This was apparent in data from crosses between plaque-forming strains and normal strains in which only 50% or less of progeny were plaqued. Nonplaqued progeny from such crosses were able to carry virus at a reduced titer and were thus potentially infectious. This was demonstrated by crossing a nonplaqued strain with an uninfected strain known to be sensitive to virus. Although lysis occurred in neither parent, several progeny from this cross were plaqued. The nonplaqued parental strain of this cross apparently transmitted the virus and carried a gene for resistance (s^+) to the expression of plaques. The gene is a nuclear gene and is present in most strains of *S. commune*.

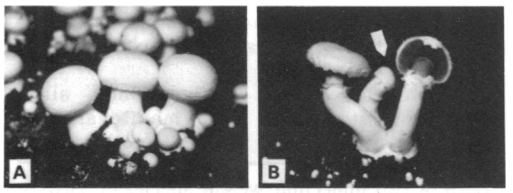


FIG. 6. *Disease symptoms of virus in Agaricus bisporus. A, Normal mushrooms in cultivation. B, Abnormal mushrooms infected with virus. Characteristic symptoms are the scattered production of mushrooms with distorted morphology. Mushroom caps are stunted (arrow) and open prematurely; stipes are often twisted and narrow (as shown) or swollen (not shown). (Magnification one-third actual size; photographs courtesy of W. Gerner, Butler County Mushroom Farm, Inc., West Winfield, Pa.)*

Thus, four phenotypes of *S. commune* can be identified through genetic analysis with regard to the presence of a specific virus and the ability to form plaques. Strains are either sensitive and contain virus ($s^-Sc\text{-}1^+$), sensitive and lack virus ($s^-Sc\text{-}1^-$), resistant and contain virus ($s^+Sc\text{-}1^+$), or resistant and lack virus ($s^+Sc\text{-}1^-$). Only strains of the first type ($s^-Sc\text{-}1^+$) form plaques.

Determination of plaque formation in *S. commune* differs somewhat from the determination of virulence by killer factors in two other

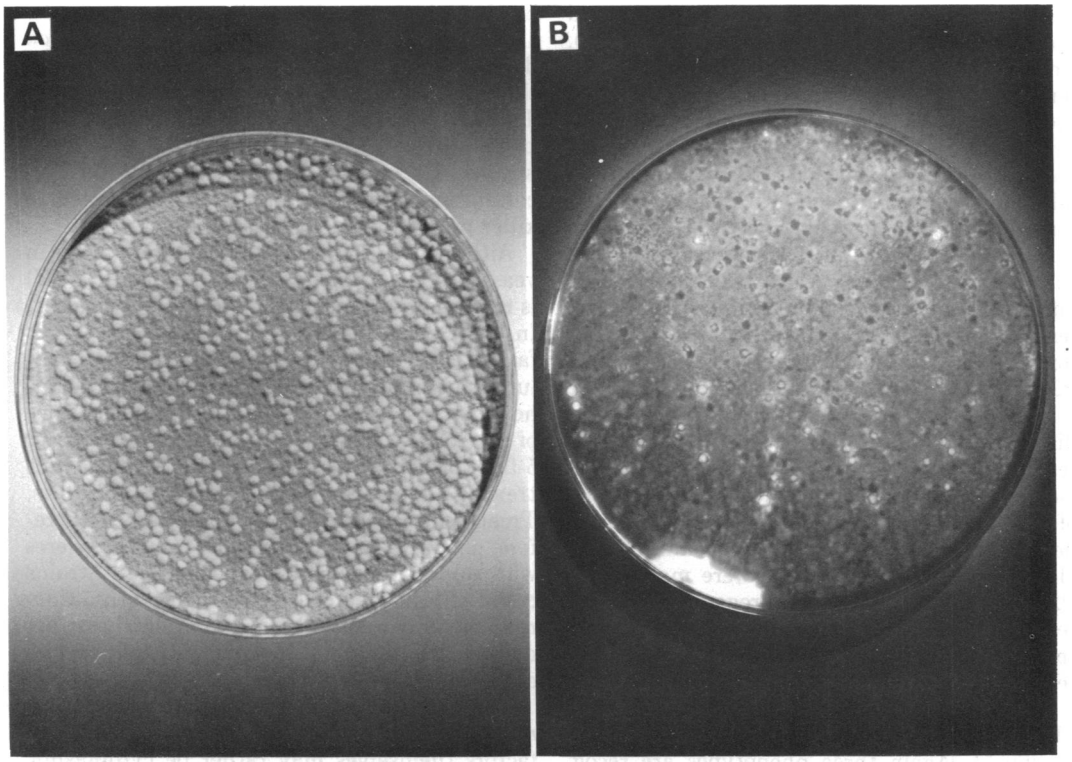


FIG. 7. *Lytic plaque formation in Penicillium chrysogenum. A, Early plaque formation indicated by erumpent patches of white, asporogenic mycelia on the surface of the culture. B. Late plaque formation observed by transmitted light. Plaques, thus observed, are either clear, turbid, or turbid with clear centers.*

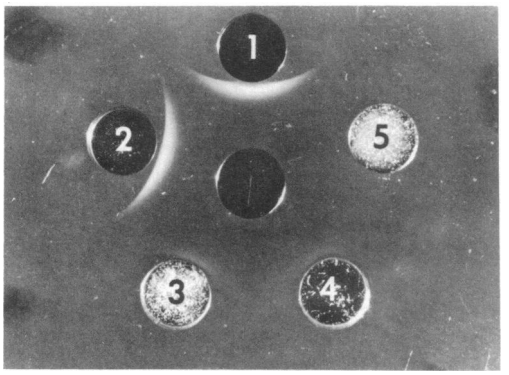


FIG. 8. *Immunodiffusion assay of virus from strains of Penicillium chrysogenum: rabbit antiserum to purified virus (center), purified virus (1), control strain (2), strains treated to reduce virus titer (3, 4, 5). Heat-treated strain (5) apparently cured of virus.*

sexually reproducing fungi, *Ustilago maydis* and *Saccharomyces cerevisiae*.

Killer Systems of Ustilago maydis and Saccharomyces cerevisiae

Some strains of *U. maydis*, a smut fungus of corn, produce a toxic protein lethal only to sensitive strains of the fungus (50, 88, 171). Resistance to killer toxin is somehow conferred upon strains that harbor a virus (51, 52, 175). This virus, *Um*-1, is spherical, 41 nm in diameter, and contains dsRNA. The virus as well as the ability to synthesize toxin are cytoplasmically inherited factors. Tests involving heterokaryosis have revealed that strains of *U. maydis* fall into three phenotypes with regard to production of toxin and sensitivity to it (Table 5). Strains designated P1 synthesize toxin but contain virus and are resistant. P3 strains do not produce toxin but do contain virus and are resistant. Finally, P2 strains do not produce toxin and lack virus. Sensitivity or resistance of P2 strains depends on whether they carry the alleles s^- or s^+ at a chromosomal locus. P3 is distinguishable from P2s^+ by a simple breeding test. If P3 (unknown) is mated with P2s^- (sensitive), all progeny should be resistant and of P3 phenotype, a result expected from a heterokaryon test for cytoplasmic inheritance. However, if P2s^+ (unknown) were mated with P2s^-, then only 50% of progeny would be resistant, a result consistent with Mendelian segregation of the chromosomal allele (s^+) for resistance.

The killer system of *S. cerevisiae* is comparable to that of *U. maydis* in several respects (Table 5). Again three phenotypes are recognized (i.e., killer, neutral, and sensitive), and the system is genetically controlled by at least two cytoplasmic determinants and one nuclear gene (17, 19, 39, 76, 162, 192, 207, 219). Killer cells synthesize a toxic protein lethal only to sensitive cells, and neutral cells neither kill nor are killed.

TABLE 5. *Killer systems of Ustilago and yeast*

Ustilago maydis	*Saccharomyces cerevisiae*
P1: produces toxin, contains virus	Killer: produces toxin, contains dsRNA complex[a]
P3: no toxin, contains virus	Neutral: no toxin, contains dsRNA complex
P2: no toxin, lacks virus	Sensitive: no toxin, lacks dsRNA complex[b]
s^+ = chromosomal allele for resistance to toxin	*M* = chromosomal allele for maintenance of killer factor

[a] Both of two molecular weight species of dsRNA, 2.5×10^6 and 1.4×10^6, are present in killer and neutral strains of yeast (17).

[b] Sensitive strains of yeast either lack both species of dsRNA or possess only the larger of the two species (17).

An unusual complex of two molecular weight species of dsRNA, presumably of viral origin, has been identified from killer and neutral cells (16–18). Sensitive cells, in general, either lack dsRNA completely or contain only the larger of the two dsRNA species present in killer cells. The sensitive strains that possess dsRNA have been derived experimentally from killer strains. Except for these isolates, a positive correlation between dsRNA and resistance to killer protein exists in yeast.

Although the inheritance of killer factor in yeast is cytoplasmic, a chromosomal allele (*M*) is required for maintenance of the killer factor. In cells with the alternate allele (*m*), killer factor is not duplicated and is eventually diluted out from a growing culture. Killer factor, however, can be eliminated even in the presence of *M* by treatment with cycloheximide (76) or 5-fluorouracil (17, 18, 154). Killer strains, thus treated, may become sensitive nonkillers. Treatment with specific antimetabolites, therefore, not only removes killer factor but interferes with some factor for resistance as well. Strains cured of killer factor by treatment with 5-fluorouracil either lack dsRNA completely or are deficient for the small-molecular-weight species of dsRNA (17, 90).

There is no evidence in either yeast or *U. maydis* that killer factors per se are viruses. The factors themselves may rather be cytoplasmic determinants of some other kind. There is, however, suggestive evidence that a virus in the case of *U. maydis* and a dsRNA complex in the

case of yeast endow their respective hosts with resistance to killer substances. In yeast, moreover, the smaller of two molecular weight species of dsRNA is somehow necessary for killer determination. Bevan and co-workers (17) have further observed the presence of icosahedral virus particles both in killer strains and in those sensitive strains which retain the larger-molecular-weight species of dsRNA. No virus particles were seen in sensitive strains which lack dsRNA. The virus particles have not, however, been isolated, and it is not known if they contain dsRNA.

Fungal Viruses and Host Metabolism

A wide variety of uncommon or secondary metabolites are synthesized by fungi (20, 68, 84, 135, 183). It is often assumed that compounds of this nature represent inborn errors of metabolism. However, the genetic basis for synthesis of secondary metabolites by fungi is largely undetermined. Those secondary metabolites with biological activity, antibiotics and toxins, have been studied preferentially, and it has been suggested that synthesis of such compounds might be related to the presence of virus (138). Evidence obtained thus far from studies with fungal viruses, as discussed below, indicates that viruses are not involved, at least not directly, in the synthesis of secondary metabolites. Studies, however, have been few and cursory.

All strains of *P. chrysogenum*, regardless of virus titer, retain the ability to synthesize penicillin (137). A strain of *Penicillium notatum*, another penicillin-producing fungus, synthesizes penicillin in the apparent absence of virus (209). From these observations, however, one cannot rule out the possibility that virus in an unrecognized or integrated state directs antibiotic synthesis. Some strains of *P. notatum* do contain virus (100), and another antibiotic-producing fungus, *Cephalosporium chrysogenum*, harbors virus at a very low titer (49, 135).

A negative relationship between virus and the synthesis of aflatoxin was implied from studies with two species of *Aspergillus* (149). Virus particles 30 nm in diameter were observed in a non-aflatoxin-producing strain of *A. flavus*. However, virus particles were not seen in aflatoxin-producing strains of *A. flavus* or in a strain of *A. parasiticus*, which produces abundant aflatoxin.

Strains of *P. brevicompactum* and *P. stoloniferum* synthesize mycophenolic acid, a known antimetabolite of guanosine (41, 53). An inverse relationship apparently exists between viral titers and the levels of mycophenolic acid produced by specific strains of these two molds (53, 53a). This relationship implies that synthesis of mycophenolic acid somehow inhibits replication of viruses in these *Penicillium* species. Borré and co-workers (25), in unrelated experiments, have indeed shown that the addition of mycophenolic acid to growth medium inhibits the formation of lytic plaques by virus-infected cultures of two other *Penicillium* species, *P. citrinum* and *P. variabile*. Conceivably, mycophenolic acid as well as certain other unusual compounds synthesized by fungi, by virtue of their antiviral activity, could afford some natural protection for fungi against viruses.

Chang and Tuveson (43) have observed spontaneous variation in virus titer among isolates from a culture of *A. foetidus*. Isolates auxotrophic for proline occurred at high frequency in this culture, and these isolates apparently have less virus than prototrophic mycelia. Chang and Tuveson have suggested that high virus titer in *A. foetidus* might somehow suppress a native requirement for proline.

Strains of *P. stoloniferum* lacking virus are apparently deficient for galactosamine content of cell wall (34). One strain of *P. stoloniferum* infected with virus was shown to have between 18- and 45-fold more cell wall galactosamine than uninfected strains. As Buck and co-workers have suggested (34), this difference may somehow be related to infectivity and transmission of virus *Ps*-1. This relationship is not understood at the present time.

Fungal Viruses and Plant Pathology

Many fungi are pathogenic to higher plants, and viruses are associated with several of these fungi (5, 7, 29, 31, 54, 74, 104, 175, 193, 200, 221, 223). The pathogenicity of these fungi may, in certain instances, be influenced by virus. The interrelationship of virus and fungus with regard to plant pathology will depend upon whether the virus is a pathogen of the fungus or merely transmitted by the fungus to a higher plant. If the virus is virulent against the fungus, then it should antagonize pathogenicity promoted by the fungus. However, if the virus exhibits virulence against the higher plant and is noninfectious toward the fungus, then a fungus that carries such a virus is a vector which simply transmits pathogenicity. In either case, pathogenicity to the higher plant is influenced by the virulence of the virus. So far, no virus is known which can infect and multiply in both a phytopathogenic fungus and a higher plant.

Viruses and fungi are often synergistic in producing disease symptoms among plants (73, 159, 166, 178), and in some instances plant viruses may actually be carried on the surface of fungal spores (54, 200). Such viruses, however,

do not qualify as fungal viruses. Only those viruses that are present and multiply in the fungal cell qualify. To date, fungal viruses have been identified in at least ten phytopathogenic fungi—*Helminthosporium victoriae* (26), *H. oryzae* (194), *H. maydis* (31), *Sclerotium cepivorum* (113, 116), *Puccinia graminis* (158, 175), *Colletotricum lindemuthianum* (175, 176), *Diplocarpon rosae* (29), *Thanatephorus cucumeris* (26), *Piricularia oryzae* (74, 195, 221), *Gaeumannomyces* (*Ophiobolus*) *graminis* (128, 132, 176, 177), *Gonatobotrys* sp. (193a), *Periconia circinata* (65), and an *Aphelidium* species (182).

Twelve strains of *Helminthosporium maydis*, the Southern corn blight fungus, were screened for the presence of virus (26, 31). Virus particles were present only in strains which caused severe disease symptoms in corn. Particles were not found in mildly pathogenic strains of this fungus.

In *Periconia circinata*, a fungus associated with root rot and crown rot of sorghum, virus particles can be found in nonpathogenic as well as pathogenic strains (65).

The fungus *Gaeumannomyces* (*Ophiobolus*) *graminis* causes root rot in wheat. Certain strains of this fungus infected with a virus 29 nm in diameter grow slowly, sporulate poorly, and are weakly pathogenic (7, 128, 132, 193). Conversely, strains without virus grow normally, sporulate well, and are strongly pathogenic. Lemaire and co-workers in France (133) have demonstrated that virus can be transmitted from infected to normal strains of *G. graminis*. Normal strains, once infected, became less pathogenic. These preliminary field experiments constitute the first attempt at biological control of a fungal disease by a virus.

Rawlinson and co-workers in England (176, 177) also studied the phytopathogenicity of virus-infected strains of *G. graminis* but obtained somewhat different results. In their studies, growth rate, sporulation efficiency, and pathogenicity are determined largely by genetic determinants other than virus. The viruses in their strains are relatively latent. Two kinds of virus, one 27 nm in diameter and one 35 nm in diameter, were identified in strains of *G. graminis* investigated by these British workers. Either one or both kinds of virus were present in virus-infected strains. Strains with both kinds of virus were generally less pathogenic to wheat than strains without virus. However, strains containing either one of the two kinds of virus were often more pathogenic to wheat than strains lacking virus.

In agreement with the French workers, Rawlinson and co-workers were able to confirm that viruses of *G. graminis* could be transmitted between compatible strains and were not carried by the ascopores of this sexually reproducing fungus.

Strains of *G. graminis*, like strains of *S. commune* and *P. chrysogenum*, can apparently differ for resistance or susceptibility to virus. In *S. commune*, and perhaps also in *P. chrysogenum*, at least one nuclear gene confers resistance to plaque formation by virus. Only about 2% of natural isolates of *S. commune* form plaques when infected by virus (Y. Koltin, personal communication), and plaque formation in *P. chrysogenum* has been observed only among mutants obtained in the laboratory (137). Genes that control susceptibility of fungi to viruses are apparently mutable and appear to vary even among strains of the same fungal species.

In the evolution of fungi there have undoubtedly been selective pressures for tolerance to viral infection. This conclusion seems unavoidable, since so many examples of latent viruses have now been found in fungi. The genetic basis for this tolerance to virus remains largely undetermined.

TRANSMISSION OF FUNGAL VIRUSES

Considerable reference has already been made in this review with regard to transmission of fungal viruses. Transmission of fungal viruses, in general, entails exchange of cytoplasm between fungal cell lines. Cytoplasmic exchange and viral transmission are achieved by fungi through heterokaryosis, a phenomenon which is common and unique to fungi. Heterokaryosis involves plasmogamy between cells that are compatible and thus permits the transmission of fungal viruses without extensive cellular lysis or release of infectious free virus. Incompatibility between fungi would restrict the spread of viruses through heterokaryosis and thereby effectively delimit the host range of a fungal virus. Heterokaryon formation in fungi is under strict genetic control. The genetic systems that control heterokaryosis and incompatibility in fungi have been reviewed recently (70, 134).

Transmission of Viral Diseases in the Cultivated Mushroom

The transmission of viruses which infect *A. bisporus* has been extensively studied and recently reviewed by Hollings and Stone (99, 100) and by Dieleman-van Zaayen (59). Viral transmission has been demonstrated repeatedly by inoculating an uninfected culture with either spores or cellular fragments from an infected strain. Mushroom viruses proliferate rapidly

and spread radially from an initial locus of infection (81, 82, 95, 130, 181). Disease symptoms include reduced hyphal growth and the production of abnormal mushrooms. These symptoms accompany the spread of viruses through hyphae.

Infection with virus can be brought about artifically by injecting virus preparations into mushroom primordia (98). Mycelia beneath the site of injection become infected, and mushrooms produced subsequently by these mycelia carry virus. Infection does not occur if primordia are simply exposed to virus preparations without injection.

Studies on the transmission of mushroom viruses indicate a requirement for either cytoplasmic exchange through heterokaryosis or infection of mushroom tissue through artificial injection.

A. bisporus has recently proven to be amenable to genetic analysis (153, 172) which should make it possible to study further the transmission and biology of mushroom viruses. Unfortunately, very little is known about the biochemical and biophysical properties of *A. bisporus* viruses.

Transmission of Viruses in Fungi Through Heterokaryosis

The first experiments to confirm that viruses in fungi could be transmitted through heterokaryosis were conducted by Lhoas (139, 140). These experiments involved genetically marked strains of two fungi, *P. stoloniferum* and *A. niger*. Strains either carried separate nutritional (auxotrophic) markers or could be distinguished by spore color markers. A heterokaryon was formed between two auxotrophic strains of *A. niger*; one strain was infected with virus and the other strain was uninfected. Single spore progeny recovered from this heterokaryon, regardless of the marker carried, were infected with virus. The virus, however, was not transmitted if the two auxotrophic strains were simply co-propagated without heterokaryosis. A second heterokaryon was synthesized by Lhoas between two strains of *P. stoloniferum*, a white-spored uninfected strain and a green-spored strain containing *Ps*-1 virus. Four out of six white-spored progeny derived from this heterokaryon were infected with *Ps*-1 virus.

Viral transmission through heterokaryosis has now been demonstrated in other fungal species—*Ustilago maydis* (216), *Schizophyllum commune* (112), *Gaeumannomyces graminis* (133, 177), *Penicillium claviforme* (152), and *P. chrysogenum* (Nash and Lemke, unpublished results). Cytoplasmic determinants of a probable viral nature have been transmitted through heterokaryosis in certain other fungi—*Helminthosporium maydis* (144), *Aspergillus glaucus* (103), *Aspergillus amstelodami* (42), *Podospora anserina* (151), and *H. victoriae* (143).

Infection of Fungal Protoplasts by Purified Virus

Although heterokaryosis appears to be a common vehicle for the transfer of fungal viruses, it seems certain that viruses can infect fungal cells under other circumstances. It is not now known if free viruses or their nucleic acids can traverse the fungal cell wall, but there is evidence that virus infection can take place in fungi across the cell membrane. Uninfected protoplasts of *P. stoloniferum* incubated in the presence of purified *Ps*-1 virus became infected (141). Initial titers of virus in the regenerating protoplasts were low, but titers increased with subsequent growth. Uninfected cells of *P. stoloniferum* with intact cell walls were not infected when mixed with *Ps*-1 virus. Similar experiments on the infection of host protoplasts have been conducted with purified virus from *P. chrysogenum* (167).

Viruses from *P. stoloniferum* and *A. niger* can apparently infect cells of *S. cerevisiae* (23, 142). This infection, however, takes place under rather specialized conditions, during the mating of haploid yeast cells. Yeast cells were not infected by purified viruses in the absence of zygote formation. The mating process of yeast involves plasmogamy with a localized dissolution of cell walls between adjacent and compatible cells. The breakdown of the cell wall at this time apparently affords free virus the opportunity to infect incipient yeast zygotes. In 1967, Kovács and Bucz claimed that yeast protoplasts as well as intact yeast cells could be infected with an RNA-containing mammalian virus, encephalomyocarditis virus (115).

Host Specificity of Viruses in Fungi

Certain of the dsRNA-containing viruses present in fungi have been examined serologically (Table 2). Viruses present among species of *Penicillium* are, in general, serologically distinct (12). However, *Pc*-1 virus from *P. chrysogenum* is related to *Pb*-1 virus from *P. brevicompactum* (218). Moreover, the virus present in *Diplocarpon rosae* is serologically related to the *Ps*-1s virus of *P. stoloniferum* (29), and viruses from *A. niger* are immunologically related to the electrophoretically fast and slow viruses of *A. foetidus* (13).

Fungi, in addition to harboring their own viruses, may be susceptible to infection by plant and animal viruses (Table 6). Yeast cells appear

to be nonselective hosts for a number of unrelated viruses. Certain mammalian viruses added to growing cultures of *S. cerevisiae* and *Candida albicans* can apparently multiply (114-117). The increase of polyoma virus in these experiments was measured by several assays including a conventional assay for plaque formation in mammalian tissue culture (117). In the same series of experiments, Kovaćs and Bucz reported that yeast cells were also susceptible to infection by RNA derived from poliovirus (115). Lindegren and co-workers (146) as early as 1962 reported that viruses similar in morphology to tobacco mosaic virus and to adenovirus could be found in lysates of abnormal yeast cells. Tobacco mosaic virus has recently been transmitted to *S. cerevisiae* and propagated at low titer through several generations of yeast cells (146).

Tobacco mosaic virus (TMV) has been reported to be associated with at least three other fungi—*Erysiphe graminis* (163), *Sphaerotheca lanestris* (163), and *Pythium sylvaticum* (32, 33, 213). There is no evidence at this time to indicate that TMV invades cells of *Erysiphe graminis* or *Sphaerotheca lanestris*. Nienhaus (163) and, more recently, Yarwood and Hecht-Poinar (223) have simply shown that spores of these two fungi transmit disease symptoms characteristic of TMV to plants. Attempts to infect and propagate TMV in *Pythium sylvaticum*, however, have been made (32, 33, 213). These experiments, like those discussed above with reference to yeast cells, do indicate some multiplication of TMV in growing cultures of a fungus.

Fungi may represent a reservoir in nature for both plant and animal viruses. Exactly how such viruses might infect fungal cells under natural conditions is not now known.

TABLE 6. *Nonfungal viruses associated with fungi*[a]

Fungal species	Virus	Reference(s)
Candida albicans	Polyoma virus	117
Coleosporium asterum	Tobacco mosaic virus	223
Coleosporium madiae	Tobacco mosaic virus	223
Erysiphe graminis	Tobacco mosaic virus	163, 223
Olpidium brassicae	Lettuce big vein virus	77, 86
	Tobacco necrosis virus	198-200
	Tobacco stunt virus	91, 93
Olpidium cucurbitacearum	Cucumber necrosis virus[b]	54
Penicillium brevicompactum	Bacteriophages	205
Polymyxa graminis	Wheat mosaic virus	72
Pythium sylvaticum	Tobacco mosaic virus	32, 33, 213
Saccharomyces cerevisiae	Encephalomyocarditis virus	114
	Tobacco mosaic virus	46
	Polyoma virus	117
	Polio virus	115
Sphaerotheca lanestris	Tobacco mosaic virus	163, 223
Spongospora subterranea	Mop-top virus	104
Synchytrium endobioticum	Potato virus X	164
Uromyces fabae	Tobacco mosaic virus	223
Uromyces phaseoli	Tobacco mosaic virus	223

[a] Experimental evidence for replication of nonfungal viruses in fungi is available for only three of the species listed here, i.e., *Saccharomyces cerevisiae*, *Candida albicans*, and *Pythium sylvaticum* (see text). The viruses in all other cases are apparently carried on the surface of fungal cells or have not been shown to multiply within growing cells of fungi.

[b] According to Dias (54), cucumber necrosis virus is present within resting spores of *Olpidum cucurbitacearum*.

Reinfection Experiments with Penicillium chrysogenum

Essentially all of Koch's postulates have been considered and have been met in studies involving the virus from *P. chrysogenum*. This virus has been isolated and characterized biophysically in several laboratories (35, 161, 215). Biological characterization of the virus has followed (137, 161) with the demonstration that certain virus-infected strains form plaques (Fig. 7) and that strains can be cured of virus infection either by thermotherapy or by treatment with cycloheximide. Strains freed of virus no longer form plaques (137).

Reinfection experiments involving heterokaryosis have now been conducted with strains of *P. chrysogenum* (Nash and co-workers, unpublished results). A heterokaryon was formed between a white-spored, methionine-requiring mutant freed of virus and a yellow-spored, cysteine-requiring mutant infected with virus. From this prototrophic heterokaryon, 30 white-spored and 7 yellow-spored strains were isolated, and a single green-spored diploid strain was recovered. Each strain was examined for virus and the ability to form plaques. Viral titers were measured by immunoprecipitation. All strains regardless of spore color were infected with virus and routinely formed plaques.

CONCLUDING REMARKS

Fungal virology, to date, has been largely descriptive. Viruses are now recognized as common among fungi, but the biological significance of this is not now apparent. The presence of virus might be expected to influence profoundly the biology and evolution of fungi. At this time, it seems appropriate not simply to review the subject but to make certain predictions concerning the relationship between fungus and phage.

First and foremost, fungal viruses appear to be more latent than lytic, a feature that accounts largely for their belated discovery. Virus particles occur in fungi, often at high titer, without extensive or predictable lysis of fungal cells. Indeed, the majority of fungal viruses described thus far appear to be latent. Ultrastructural examination of infected cells reveals that virus particles in older cells are present in membrane-bound vesicles of otherwise normal cytoplasm. Through such intracellular partitioning of virus, fungal cells seem to accommodate infection by virus relatively well. Although infected fungal cell lines often grow slowly or develop abnormally, they do not regularly lyse.

Resistance of fungi to lysis by viruses is not, however, absolute. Viral plaque formation occurs in some strains of *Penicillium chrysogenum* and in *Schizophyllum commune*. In *P. chrysogenum* lysis is limited to regions of a colony which exhibit rapid but undifferentiated growth. In *S. commune*, and perhaps also in *P. chrysogenum*, the expression of plaque formation is determined by at least one nuclear gene. Plaque formation in these fungi is not comparable to that observed in bacteria, since plaques are not formed regularly by cells that produce virus particles.

Extensive lysis and external release of virus would not be required for viral transmission in fungi. The fungi, including many asexual fungi, exhibit plasmogamy through heterokaryon formation. Formation of the heterokaryon involves localized lysis between adjacent and compatible cells and involves cytoplasmic exchange. Heterokaryosis is characteristic of fungi and is genetically controlled through systems of incompatibility. Systems of incompatibility determine the breeding potential of fungi and would thus influence the spread of viruses among fungal populations.

Fungi as a group are paradoxical with regard to breeding potential. Against a background of varied sexual cycles, fungi often have a tendency to inbreed. Hybridization is extremely uncommon in fungi, and even within a species sterility barriers often exist to promote inbreeding. Many fungi, moreover, are self-fertile, and this condition (homothallism) is in some fungi clearly derived. The selective pressures for inbreeding in fungi are largely undetermined. The deuteromycetes or imperfect fungi represent about one-third of the known fungi, and these fungi have for some reason lost sexuality. Inbreeding and loss of sexual competence would, of course, effectively limit the spread of viruses through heterokaryosis. Viruses or, more specifically, the threat of virulence by virus could constitute a major selective force for inbreeding and apomixis in fungi.

Restrictive breeding tendencies can be brought about in fungi by ecological factors, since related fungi sometimes diverge into extremely narrow ecological niches. Fungi pathogenic to higher plants, for example, can exhibit extreme host specialization as one pathogenic species may become divided into several physiological races. The selective advantage for host specialization by pathogenic fungi may be related to inhibition of viral transmission.

The importance of viruses associated with plant pathogenic fungi is at least twofold. First, several fungi have been implicated as vectors for pathogenic plant viruses. Secondly, virulence on the part of a fungal virus may curtail phytopathogenicity by a fungus. Studies with

some strains of *Gaeumannomyces graminis* have already demonstrated that infection by virus influences pathogenicity of that fungus toward wheat. Further study of fungal viruses should contribute significantly to the understanding and potential use of viruses for biological control of fungal diseases in higher plants.

The relationship between viruses and fungal metabolism is at this time especially ill defined, and virtually nothing is known about the genetic cycle of a fungal virus. Many cytoplasmically inherited characteristics of fungi may eventually prove to be viral in origin. The killer systems of yeast and a smut fungus apparently involve viruses, but in these two systems other genetic determinants are involved as well.

Thus far, the fungal viruses studied extensively all have proven to be dsRNA viruses. It is not likely that such viruses engage in genetic determination beyond their replication. To date, no one has described a DNA virus in a fungus amenable to genetic analysis. Such a system would provide a model experimental system whereby to study the genetic influence of a virus upon the eukaryotic cell.

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